DOCUMENT 42

Journal of Children's Health: Vol. 2, No. 1, pp. 53–76 (2004)

ISSN: 0888-0018 print / 1521-0669 online
DOI: 10.1080/15417060490447378

Quantitative Evaluation of Perfluorooctanesulfonate (PFOS) and Other Fluorochemicals in the Serum of Children

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ABSTRACT

Perfluorooctanesulfonyl fluoride (POSF)–based materials include surfactants, paper and packaging treatments, and surface (e.g., carpet, upholstery, textile) protectants. A metabolite, perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$), has been identified in the serum and liver tissue of nonoccupationally exposed adults and wildlife. Results from several repeat-dose toxicological studies consistently demonstrate that the liver is the primary target organ with an apparent threshold for the toxic effects of PFOS that can be expressed in terms of cumulative dose or body burden. The purpose of this study was to characterize the distribution of PFOS and six other fluorochemicals in 598 serum samples obtained from a multi-center study of children (ages 2–12) diagnosed with group A streptococcal infections. Using high-pressure liquid chromatography tandem mass spectrometry methods, serum PFOS concentrations ranged from 6.7 ppb (ng/mL) to 515 ppb (geometric mean 37.5 ppb, 95% CI 36.0–39.1) with an estimate of the 95th percentile (i.e., upper tolerance limit) of 89 ppb (upper 95% confidence limit 97 ppb). Serum perfluorooctanoate (PFOA) concentrations were approximately an order of magnitude lower than PFOS. Unlike comparable adult data reported elsewhere for PFOS and PFOA, children had substantially higher estimates for the 95th percentile for perfluorohexanesulfonate (65 ppb) and N-methyl perfluorooctanesulfonamidoacetate (12 ppb) (upper 95% confidence limits of 81 ppb and 15 ppb, respectively). The reasons for these dissimilarities in a subgroup of children remain to be determined. Different exposure and activity patterns between children and adults should be considered.

Key Words: children, biomonitoring, fluorochemicals, perfluorooctanesulfonate, perfluorooctanoate, perfluorohexanesulfonate.

INTRODUCTION

Perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$), produced by an electrochemical fluorination process, was used as the basic building block by the 3M Company (3M) to create unique chemistries by further reactions with functionalized hydrocarbon molecules. Major applications of the resulting fluorochemical products have included surfactants, paper and packaging treatments, and surface (e.g., carpets, upholstery, textiles) protectants. Depending upon the specific functional derivatization or the degree of polymerization, such POSF-based products may degrade or metabolize, to an undetermined degree, to perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$), a stable and persistent end-product that has the potential to bioaccumulate (3M Company 2003). In May, 2000, 3M announced that it would voluntarily cease manufacturing POSF-based materials after PFOS was found to be prevalent in many locations in human and wildlife populations (OECD 2002; Hansen *et al.* 2001; Giesy and Kannan 2001; Kannan *et al.* 2001a, b, 2002a, b, c). While not a major commercial product itself, PFOS has been used in some products, including firefighting foams. Although not fully understood, the pathways leading to the presence of PFOS in human tissue include release to the environment of POSF-derived materials or precursor molecules in the waste streams generated from the manufacturing processes, supply chain operations, and/or consumer use (3M Company 2003; OECD 2002; Hansen *et al.* 2002; Martin *et al.* 2002). Following the company's voluntary decision to cease production, the U.S. EPA adopted regulations regarding any future manufacture or import of POSF-derived chemicals (U.S. EPA 2002).

Using high-pressure liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESMSMS), PFOS has been detected at low parts per billion (ppb = ng/mL) concentrations in approximately 1,000 general population adult serum samples from several municipalities in the United States with geometric means generally ranging between 30 to 40 ppb (Hansen *et al.* 2001; Olsen *et al.* 2003a, b, 2004). Age and gender have not been strongly associated with PFOS concentrations in these analyses of adult blood samples (Olsen *et al.* 2003b, 2004). A limited number of European adult blood samples has provided comparable results (OECD 2002; 3M Company 2003). Depending on job, fluorochemical production employees located in Decatur (Alabama) and Antwerp (Belgium) have had geometric mean serum PFOS concentrations between 500 and 2,000 ppb (Olsen *et al.* 1999, 2003c, d). Medical surveillance of fluorochemical production employees has not consistently associated serum PFOS measurements with substantial changes in clinical chemistry, thyroid hormone, or hematology results (Olsen *et al.* 1999, 2003c). An excess of bladder cancer deaths (N = 3) was reported but its relevance to PFOS remains to be determined (Alexander *et al.* 2003).

Children's exposures to environmental chemicals from nonoccupational exposures can be different, and sometimes higher, than adults (Bearer 1995; Cohen-Hubal *et al.* 2000). These differences can be a function of a multitude of interrelated factors, which include physiology, behavior, physical activity, diet, age, sex, socioeconomic status, race, and ethnicity. POSF-based materials were used for three major consumer applications that could involve potential exposures to children from: 1) carpet and upholstery protectants; 2) textile protectants; and 3) paper and packaging protectants used in food applications. Non-product environmental exposures are also possible.

The purpose of the present study was to characterize the distribution of PFOS and six other fluorochemicals, including two precursors of PFOS related to POSF-consumer applications, in 598 serum samples obtained from a multi-center study of children (ages 2–12) who were diagnosed with group A streptococcal infections. Exposure histories of these children were not known. We then compared the present study findings to the serum fluorochemical distributions determined in adult and elderly populations, which have been reported elsewhere (Olsen *et al.* 2003b, 2004).

METHODS

Fluorochemicals

The seven analytes detected and quantified in this study were PFOS; N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA, $C_8F_{17}SO_2N[CH_2CH_3]CH_2COO^-$); N-methyl perfluorooctanesulfonamidoacetate (M570, $C_8F_{17}SO_2N[CH_3]CH_2COO^-$); perfluorooctanesulfonamidoacetate (M556, $C_8F_{17}SO_2NHCH_2COO^-$); perfluorooctanesulfonamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_7F_{15}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

PFOSAA is an oxidation product of N-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE, $C_8F_{17}SO_2N[CH_2CH_3]CH_2CH_2OH$), which was a residual found in products primarily used in paper and packaging protectant applications. M570 is an oxidation product of N-methyl perfluorooctanesulfonamidoethanol (N-MeFOSE, $C_8F_{17}SO_2N[CH_3]CH_2CH_2OH$), which was a residual found in products used primarily in surface treatment applications (e.g., carpets, upholstery, textiles). Therefore, PFOSAA and M570 can be considered markers of consumer-related exposure. Both PFOSAA and M570 can metabolize to M556 and PFOSA, which in turn can subsequently metabolize to PFOS. Unlike PFOSAA and M570, the three analytes M556, PFOSA, and PFOS are not specific to any one consumer application. PFOA and PFHS are not known to be precursors or metabolites of PFOS. PFOA was produced by 3M and others to be an emulsifier in a variety of industrial applications. PFOA was also found as a trace impurity in some POSF-based materials (Wendling 2003). PFOA may also be produced by environmental abiotic degradation of N-EtFOSE (Lange 2001) or by oxidation or metabolism of the widely used telomer-based fluorochemicals manufactured by other companies (Hagen *et al.* 1981). PFHS, the sulfonate form of perfluorohexanesulfonyl fluoride (PHSF), is a residual by-product of POSF-related

production. 3M also produced PFHS as a building block for compounds incorporated in firefighting foams and specific post-market carpet treatment applications.

Sample Collection

The serum samples analyzed in this study were initially collected as part of a large multi-center clinical trial of 1,131 children, ages 2 to 12 years, who presented with signs and symptoms of acute-onset pharyngitis and had positive throat cultures for group A streptococcal infections (Kaplan *et al.* 1998). Serum samples for the clinical trial were obtained between January 1994 and March 1995. These serum samples were chosen for the present study because of their accessibility and geographical variability. Samples were kept frozen at −20°C in plastic tubes by the University of Minnesota Department of Pediatrics prior to the 3M request for an aliquot of 0.1 mL per sample for the present study. Additional amounts were obtained for the assay reliability analysis (see below).

Because of the uncertainty regarding the population distribution of PFOS, sample size was based on tolerance intervals, which are independent of the form of the distribution (Natrella 1966). Statistical tolerance intervals have a probabilistic interpretation. The endpoints of a tolerance interval are defined as tolerance limits (lower and upper) and are the limits within which a stated proportion of a population is expected to be found with respect to some measurable characteristic. A one-sided application upper tolerance limit provides a level of confidence that a stated proportion of the population measurements will not exceed this limit. For example, the largest observation in a sample of 50 individuals of a given age and gender will be an upper tolerance limit for 95% of that specific population with a confidence level 0.90 (Natrella 1966). The distribution of age and gender sampled (N = 599) from the available 1,131 repository samples is provided in Table 1. Samples were from 23 states and the District of Columbia. Serum from one subject (8-year-old female from Texas) was not analyzed due to an insufficient quantity, yielding 598 samples for analysis. All samples were analyzed for the younger ages (2–4) because of their fewer numbers and the assumption that potential exposure experiences between these ages could be more disparate than those between children in the upper age ranges of the present study. A random sample was then chosen for the older ages.

Laboratory Assay

Comprehensive reports of the assay development, method validation, and quality control (QC) methods used in this study are available elsewhere (Tandem Labs 1999, 2001a, b, 2002). Important details of the analytical techniques and methods used, including the designation of the method limits used in the present study, are addressed summarily below and itemized elsewhere (Tandem Labs 2002). Methods were comparable to those reported for the adult (Olsen *et al.* 2003b) and elderly populations (Olsen *et al.* 2004) previously mentioned.

Briefly, the analytical method consisted of a liquid:liquid extraction procedure followed by evaporation and reconstitution of the extract residue with 20 mM

Table 1. Distribution of age and gender of children sampled for fluorochemical analyses from the N = 1,131 repository samples[1,2]

Age	Male	Female	Total (% of N)
2	9	18	27 (100)
3	26	25	51 (100)
4	45	36	81 (100)
5	40	40	80 (66)
6	40	40	80 (55)
7	30	30	60 (37)
8	30	30[3]	60 (46)
9	20	20	40 (30)
10	20	20	40 (37)
11	20	20	40 (46)
12	20	20	40 (49)
Total	300	299[3]	599[3] (53)

[1]See Kaplan *et al.* (1998).
[2]Samples (in parentheses) were from: AL (22); AZ (18); CA (47); CO (39); FL (42); ID (16); IL (2); KS (13); KY (18); MA (45); MI (9); MO (10); NC (33); NE (5); NJ (38); NM (28); NY (30); OH (33); OK (28); PA (9); TX (47); UT (26); VA (14); DC (28).
[3]One serum sample (8-year-old female) was collected but could not be used, which yielded 298 female and 598 total analyses.

ammonium acetate in water:20 mM ammonium acetate in methanol. The samples were analyzed by HPLC-ESMSMS. Quantitation of the target analytes in serum samples was performed by comparing the chromatographic peak areas for each compound to those generated in a series of extracted calibration standards prepared from control plasma determined to contain less than 4 ng/mL of any of the target analytes.

Because of the difficulty in finding suitable human sera in the United States to use for method blanks and QC samples, we obtained rural Chinese human plasma, collected in 1999, with low endogenous fluorochemical concentrations from a source within the United States. Studies designed to characterize the selectivity and the extraction efficiency for the two matrices conducted prior to the sample analysis indicated that this plasma was a suitable choice for the calibration, blank, and QC matrix samples. Evaluation of quality control samples injected during the analytical runs of the 538 samples indicated that the reported quantitative results may have varied ± 10 percent for precision and accuracy (Tandem Labs 2002).

Given the low-level presence of many of the target analytes in these biological matrices, rigorous attention to the preparation, analysis, and data interpretation of blanks, calibration standards, and QC samples was critical. The analytical system was monitored for analytical artifacts such as carryover and for potential sources of contamination.

An analysis of the reliability of the assay results was conducted after the original samples were analyzed. The laboratory was blind to the identity of these samples as they related to the original values reported. Altogether, there were 62 samples reanalyzed, representing serum from 44 children. There were strong correlations for PFOS (r = .98), PFOA (r = .96), and PFHS (r = .93), indicating a high degree of reproducibility in the analysis. Correlations were moderate for PFOSAA (r = .69) and for M570 (r = .80).

A total organic fluorine value (TOF) was calculated based on the percent of the molecular weight for each of the seven fluorochemicals that was attributed to organic fluorine. This was: PFOS (64.7%); PFHS (61.9%); PFOA (69.0%); PFOSAA (55.3%); PFOSA (64.7%); M570 (56.6%), and M556 (58.1%). This percentage was multiplied by the concentration measured for each fluorochemical and then summed across all seven fluorochemicals.

Data Analysis

Measures of central tendency applicable to lognormally distributed data (median, geometric mean) were used for descriptive analyses. In those instances where a sample was measured below the LLOQ, the midpoint between zero and the LLOQ was used for calculation of the geometric mean. A sensitivity assessment of this midpoint assumption and how it affected the calculation of the geometric mean was performed using the 10th and 90th percentile values between zero and the LLOQ for those values < LLOQ.

The log-linear relation between PFOS and PFOA was modeled as follows: $\ln[\text{PFOS}] = a \times \ln[\text{PFOA}] + c \times \text{age} + d \times \text{sex} + f \times \text{age} \times \text{sex} + g + \varepsilon$. An analogous model was used to relate PFOS to PFHS. Residuals were inspected to assure model assumptions provided reasonable fit. In order to examine the relationship between PFOS concentration and the concentration of the two other precursor molecules, PFOSAA and M570, a non-linear model was fit to the data: $\ln[\text{PFOS}] = \ln[(\text{PFOSAA})^a + (\text{M570})^b] + c \times \text{age} + d \times \text{sex} + f \times \text{age} \times \text{sex} + g + \varepsilon$ where g is the intercept and ε is the error term. This model represents an additive relation between PFOS concentration and the concentrations of the other two molecules because the hypothesized mechanisms of association—i.e., either correlated exposure sources or conversion from one molecule to another—suggested an additive rather than a multiplicative relation. At the same time, the model is consistent with the simpler models relating PFOS concentration to that of a single molecule and preserves the log-linear relationship of PFOS concentration to age and sex suggested by inspection of the residuals in these simpler models. The adjusted log-linear models were fit using maximum likelihood in the program *lm* and the adjusted nonlinear model was fit using weighted nonlinear least-squares, as implemented in the program *nls*; both are programs in S-Plus 6.0 (S-Plus 2001). The interaction term between age and sex was not a significant predictor in the models and was therefore not included in the final analyses.

To avoid making normality assumptions in these log-linear models, bootstrapping was used to form confidence intervals for the parameters (Efron and Tibshirani 1993). In this method, a large number of full-size samples of the original observations are drawn with replacement from each, of which an estimate of an upper tolerance limit is generated. The distribution of these estimates mimics the underlying sampling distribution for the original estimate assuming that the parent population looks like the sample. Bias-corrected, accelerated percentiles were used to minimize residual bias. The bias correction factor is derived by comparing empirical percentiles to bootstrap percentiles and acceleration is accomplished by partial jackknifing.

RESULTS

Children's Serum Concentrations

The overall distributions of PFOS, PFOA, PFHS, PFOSAA, M570, and TOF are displayed in Figure 1 using various scales. Although the graphs are suggestive of log-normal distributions, only the PFOS distribution met such criteria based on the Shapiro-Wilk test. This lack of lognormality may be due to the greater proportion of subjects with values <LLOQ for PFOA, PFHS, PFOSAA, and M570. Statistical analyses are not presented for PFOSA and M556 because of the fewer number of subjects whose serum concentrations exceeded the LLOQ. For PFOSA, 86% of the samples were considered <LLOQ (1.0 ppb) and the remaining 14% were at <LLOQ (2.0 ppb). For M556, 44% of the samples were <LLOQ (2.5 ppb) and 13% were <LLOQ (5.0 ppb). Of those M556 concentrations measured above the LLOQ (43%), they ranged from 2.5 to 19.1 ppb (median = 4.1 ppb). Although PFOSA and M556 are not presented in the subsequent analyses, they were included in the calculation of the TOF value assuming the midpoint between zero and the LLOQ.

Several measures of central tendency for the five fluorochemicals and TOF stratified by gender are provided in Table 2. Overall, the geometric mean concentration of PFOS was 37.5 ppb (95% CI 36.0–39.1). The PFOS values ranged from 6.7 ppb to 515 ppb. Male children had a statistically significantly ($p < .01$) higher geometric mean serum PFOS concentration compared to female children, although the absolute difference was not substantial (male children geometric mean = 40.1 ppb [95% CI 37.7–42.6] versus female geometric mean = 35.2 ppb [95% CI 33.3–37.2]). Male children also had significantly ($p < .01$) higher geometric mean serum concentrations of PFOA and PFHS than those determined for female children. There were no differences for PFOSAA and M570. The geometric mean for the calculated TOF values was 38.9 ppb (95% CI 37.2–40.7). The calculated TOF values ranged from 9.6 ppb to 803.7 ppb for all children. The TOF geometric means of male children (41.6 ppb, 95% CI 38.8–44.5) was significantly ($p < .01$) higher than female children (36.4 ppb, 95% CI 34.3–38.7).

As discussed previously, the geometric means were calculated under the assumption that, for individual serum fluorochemical values <LLOQ, the midpoint between zero and the LLOQ was assigned. For PFOS, no subject had a value <LLOQ; thus,

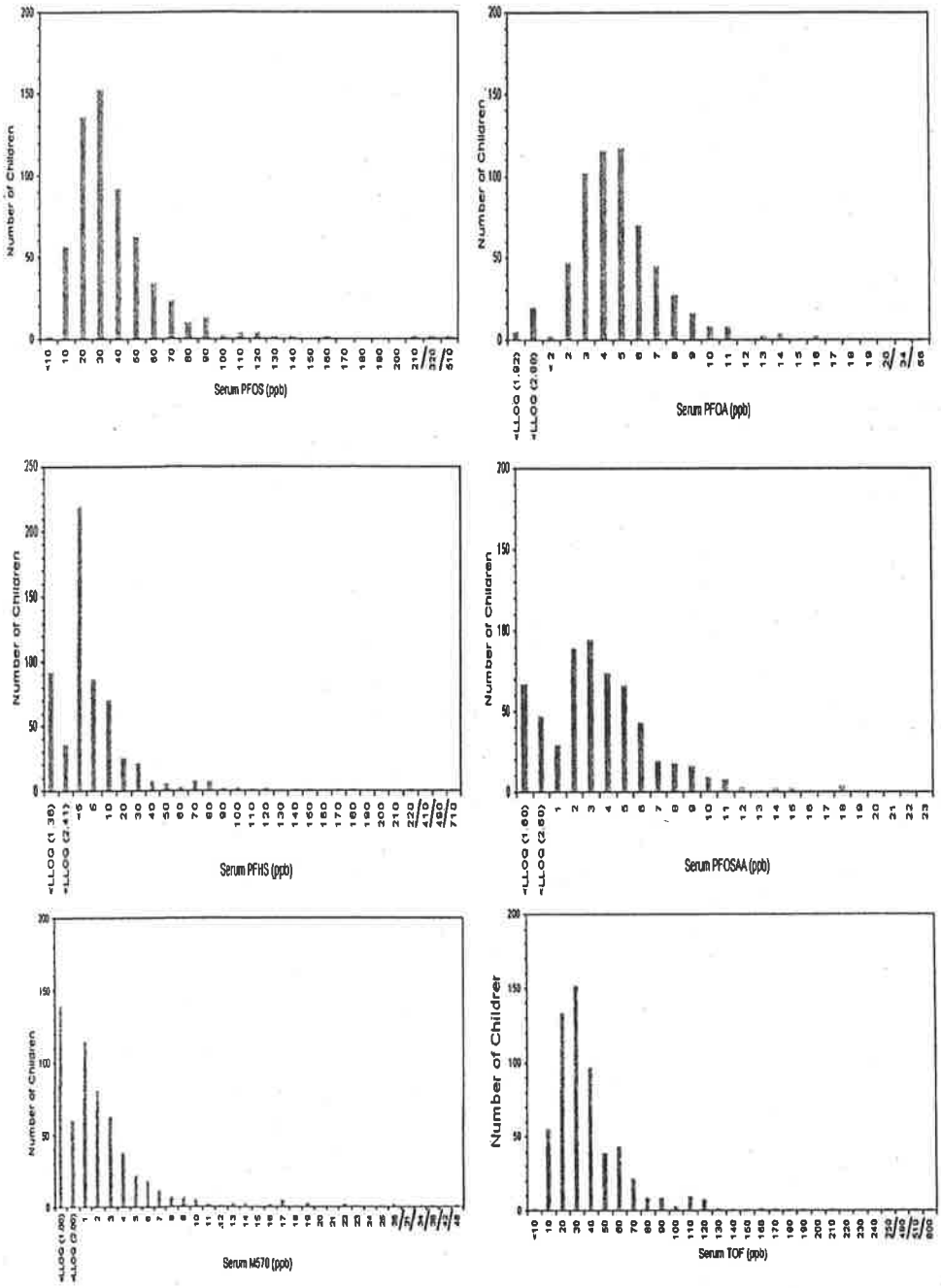

Figure 1. Distributions of serum fluorochemical concentrations and a calculated total organic fluorine value in 598 children.

Table 2. Measures of central tendency of fluorochemicals in children's serum samples

	PFOS	PFOA	PFHS	PFOSAA	M570	TOF
All Children (N = 598)						
Range	6.7–515.0	<LLOQ (1.9)–56.1	<LLOQ (1.4) –711.7	<LLOQ (1.6)–23.8	<LLOQ (1.0)–48.0	7.1–803.7
IQR*	27.6–51.0	3.8–6.7	1.6–10.8	2.1–5.6	<LLOQ (1.0)–3.8	27.1–52.1
<LLOQ (N)	<3.9 (0)	<1.9 (5)	<1.4 (92)	<1.6 (67)	<1.0 (140)	N/A
		<2.9 (20)	<2.4 (37)	<2.6 (47)	<2.0 (60)	
Cumulative 90%	70.8	8.5	35.3	8.8	7.3	78.0
Arithmetic mean	43.5	5.6	15.0	4.4	3.4	47.7
95% C.I. arithmetic mean	40.9–46.1	5.3–5.8	11.4–18.7	4.1–4.7	3.0–3.8	43.7–51.8
Median	36.7	5.1	3.8	3.7	1.8	36.7
Geometric mean	37.5	4.9	4.5	3.3	1.9	38.9
95% C.I. geometric mean	36.0–39.1	4.7–5.1	4.1–5.1	3.1–3.6	1.7–2.1	37.2–40.7
Males (N = 300)						
Range	11.4–515.0	<LLOQ (2.9)–56.1	<LLOQ (1.4) –711.7	<LLOQ (1.6)–20.7	<LLOQ (1.0)–48.0	11.1–803.7
IQR	28.7–53.9	3.9–6.9	1.9–12.2	2.0–5.8	<LLOQ (1.0)–4.2	28.0–56.3
<LLOQ (N)	<3.9 (0)	<2.9 (11)	<1.4 (34)	<1.6 (40)	<1.0 (66)	N/A
			<2.4 (20)	<2.6 (22)	<2.0 (31)	
Cumulative 90%	75.6	9.0	38.5	8.8	7.5	88.8
Arithmetic mean	47.3	5.9	19.0	4.4	3.7	53.0
95% C.I. arithmetic mean	42.7–51.9	5.4–6.4	12.2–25.8	4.0–4.7	3.0–4.3	45.6–60.4

Table 2. Measures of central tendency of fluorochemicals in children's serum samples

	PFOS	PFOA	PFHS	PFOSAA	M570	TOF
Median	39.4	5.2	4.4	3.7	2.0	39.1
Geometric Mean	40.1	5.2	5.3	3.3	2.0	41.6
95% C.I. geometric mean	37.7–42.6	4.9–5.4	4.5–6.3	3.0–3.6	1.8–2.3	38.8–44.5
Females N = 298)						
Range	6.7–165.0	<LLOQ (1.9)–18.6	<LLOQ (1.4) –170.0	<LLOQ (1.6)–23.8	<LLOQ (1.0)–38.1	7.2–217.5
IQR	27.0–46.3	3.5–6.3	<LLOQ (2.4)–10.2	2.2–5.6	<LLOQ (1.0)– 3.7	26.3–48.8
<LLOQ (N)	<3.9 (0)	<1.9 (5)	<1.4 (58)	<1.6 (27)	<1.0 (74)	N/A
		<2.9 (9)	<2.4 (17)	<2.6 (25)	<2.0 (29)	
Cumulative 90%	64.8	8.0	22.5	8.7	6.9	69.6
Arithmetic mean	39.6	5.2	11.0	4.5	3.2	42.4
95% C.I. arithmetic mean	37.2–42.1	4.9–5.5	8.4–13.5	4.1–4.8	2.6–3.7	39.2–45.7
Median	34.7	4.9	3.3	3.8	1.7	34.9
Geometric mean	35.2	4.7	3.9	3.4	1.8	36.4
95% C.I. geometric mean	33.3–37.2	4.4–4.9	3.3–4.5	3.1–3.7	1.6–2.0	34.3–38.7

*IQR = interquartile range = lower end of second quartile and upper end of third quartile.
<LLOQ = less than the lower limit of quantitation.

this assumption did not affect its calculation of the geometric mean. The number and percent of children who had values less than the LLOQs for the other fluorochemicals were PFOA (n = 25, 4%), PFHS (n = 129, 22%), PFOSAA (n = 114, 19%), and M570 (n = 200, 33%) (see Table 2). If these LLOQ values were assigned to be 10% or 90% of this range between zero and the LLOQ (instead of the midpoint), the respective range of the geometric means (95% confidence interval in parenthesis) became PFOA 4.6 ppb (4.3–4.9) to 5.0 ppb (4.8–5.2); PFHS 3.2 ppb (2.8–3.8) to 5.2 ppb (4.7–5.7); PFOSAA 2.5 ppb (2.2–2.7) to 3.7 ppb (3.6–3.9), and M570 1.1 ppb (1.0–1.3) to 2.3 ppb (2.2–2.5). These geometric mean values were not substantially different than those calculated using the midpoint between zero and the <LLOQ as presented in Table 2.

A graphical distribution (natural log scale) of the five fluorochemicals stratified by age and gender is provided in Figure 2. Analyzed as a continuous variable in simple regression models, age was negatively associated ($p < .05$) with PFOA and M570 in both males and females but not with PFOS, PFHS, or PFOSAA serum levels (data not shown). Statistical analyses by state (data not shown) were problematic because of the small sample sizes for any given age and gender combination; however, there were no substantial differences between locations.

Scatter plots (log scale) showing the correlation between PFOS and each of the four other fluorochemicals are displayed in Figure 3. PFOS and PFOA were correlated ($r = .70$), as were PFOS and PFHS ($r = 0.66$). PFOS had a lower correlation with PFOSAA ($r = .43$) and M570 ($r = .42$). Also provided in Figure 3 are scatter plots showing the correlation between PFOSAA and M570 ($r = .27$) and PFOA and PFHS ($r = 0.48$).

Both PFOSAA and M570, adjusted for age and sex, were statistically associated with PFOS in multivariable models (Table 3). PFOA and PFHS in different individual models were also associated with PFOS adjusting for age and sex.

The results from statistical bootstrap analyses conducted to provide upper tolerance limits are presented in Table 4. The upper tolerance limits represent the concentration of each fluorochemical below which the stated proportion of the population is expected to be found. Presented are the bias-corrected estimates for the 90th, 95th and 99th percent upper tolerance limits along with the upper limit (i.e., bound) from the 95% confidence interval. For example, the estimate of the 90% tolerance limit for PFOS was 71 ppb with an upper 95% confidence limit of 75 ppb. This means, in essence, that with 95% confidence we estimate 90% of children would have serum PFOS concentrations less than 75 ppb. The bias-corrected estimate of the 95% tolerance limit for PFOS was 89 ppb with an upper 95% confidence limit of 97 ppb. The estimate of the 99% tolerance limit was 141 ppb with an upper 95% confidence limit of 217 ppb.

Comparison of Serum Concentrations from Children and Adults

As seen in Figure 4, the geometric means for PFOS and PFOA in the 598 children's serum samples in the present study are similar to those reported for 645

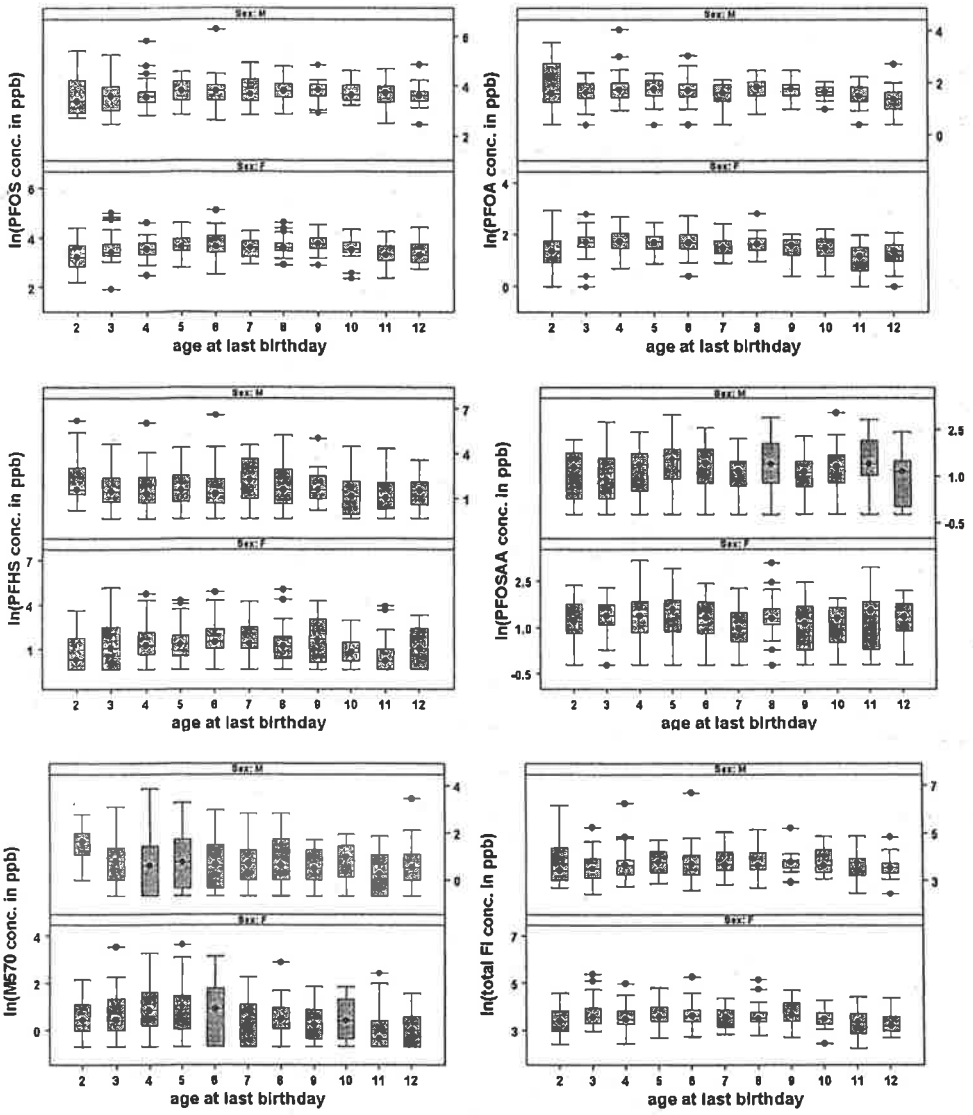

Figure 2. Box and whisker plots of serum fluorochemical concentrations by age and sex. The boxes indicate the interquartile ranges of the natural log distributions; the circle within each box is the mean; the whiskers extend to the last observation within 1.5 times the interquartile range; and the circles outside the whiskers represent observations outside the 1.5 × interquartile range.

American Red Cross adult blood donors from six geographical locations (Portland, Oregon; Los Angeles, California; Minneapolis-St. Paul, Minnessota; Charlotte, North Coralina; Hagerstown, Maryland; and Boston, Massachusetts) (Olsen *et al.* 2003b) and 238 dementia-free elderly participants of the Seattle area (Olsen *et al.* 2004).

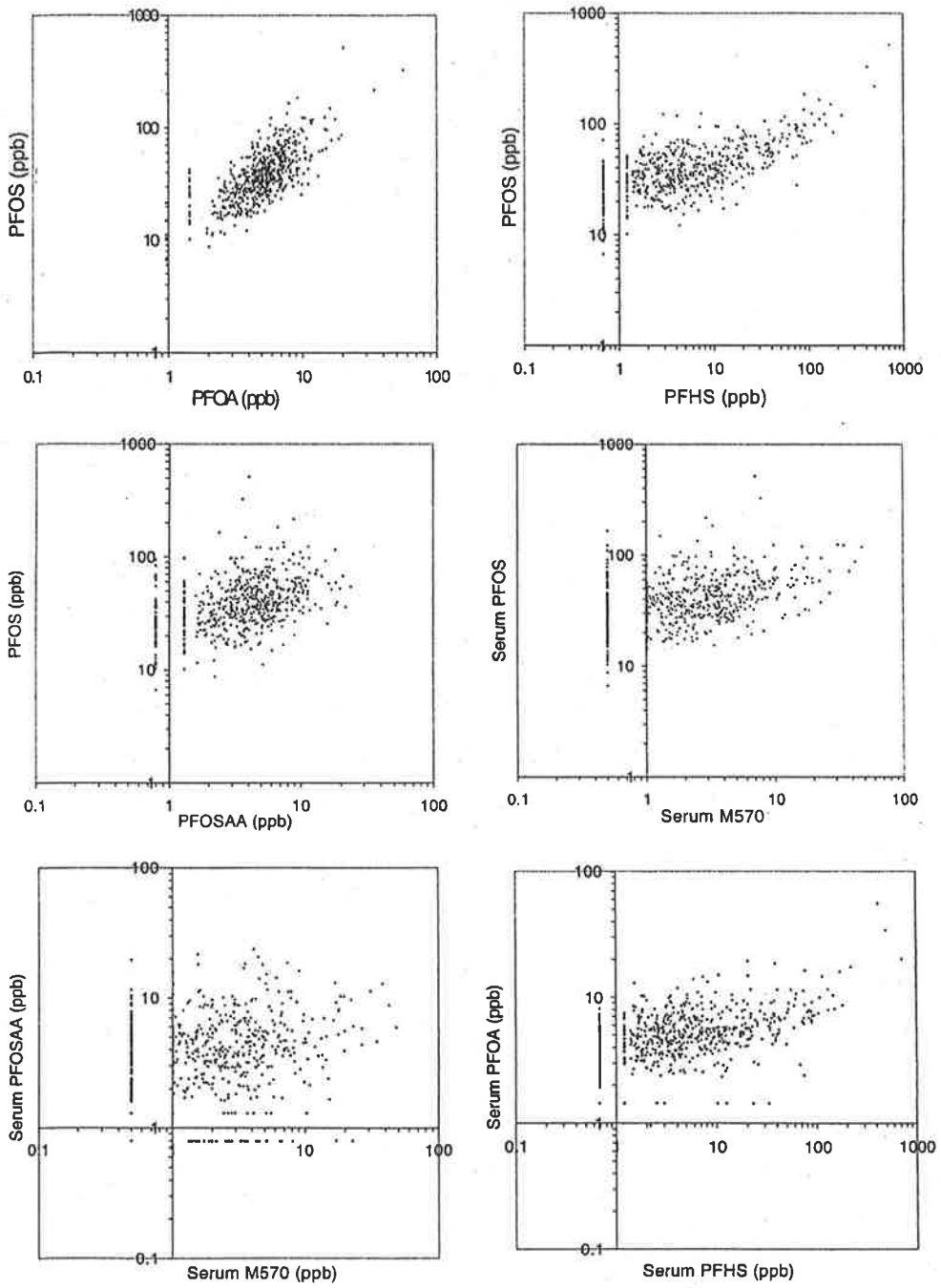

Figure 3. Scatter plots (log scale) of associations between serum fluorochemical concentrations.

Table 3. Relationship between serum concentrations of PFOS with PFOSAA and M570 (Model 1), PFOA (Model 2), or PFHS (Model 3) adjusted for age and gender

Model 1[1]	Coefficient	95% CI
Intercept	2.259	2.130–2.409
PFOSAA	0.399	0.331–0.462
M570	0.373	0.314–0.441
Age	0.016	0.004–0.029
Sex	0.117	0.049–0.181
Model 2[2]		
Intercept	2.128	1.960–2.293
PFOA	0.759	0.690–0.831
Age	0.032	0.022–0.041
Sex	0.053	(−0.003)–0.113
Model 3[3]		
Intercept	3.102	2.974–3.220
PFHS	0.242	0.219–0.267
Age	0.012	0.0007–0.023
Sex	0.051	(−0.009)–0.112

[1] $\ln [PFOS] = \ln[PFOSAA^a + M570^b] + c \times age + d \times sex + g + \varepsilon$ where g is the intercept and ε is the error term.
[2] $\ln[PFOS] = \ln[PFOA]^a + c \times age + d \times sex + g + \varepsilon$ where g is the intercept and ε is the error term.
[3] $\ln[PFOS] = \ln[PFHS]^a + c \times age + d \times sex + g + \varepsilon$ where g is the intercept and ε is the error term.

Children had higher geometric means of serum PFHS, PFOSAA, and M570 concentrations than adults but this comparison is more problematic because the LLOQs varied between studies. The assumption of a midpoint value may unduly influence a geometric mean calculation when comparing measured concentrations between studies (Griffith *et al.* 2002). The geometric mean TOF value estimated for children in the present study (39 ppb, 95% CI 37–41) was reasonably consistent with the average organic fluorine measurements of general population serum samples in the 1970s, which ranged between 25 and 45 ppb (Guy 1972; Taves *et al.* 1976; Singer and Ophaug 1979) as well as calculated total organic fluorine geometric means reported for populations of adults (32 ppb, 95% CI 30–33) (Olsen *et al.* 2003b) and the elderly (28 ppb, 95% CI 26–30) (Olsen *et al.* 2004).

Whereas the fluorochemical geometric means were not substantially different between children, adult, and elderly populations, the estimates of the 95th percentile (upper tolerance limit) indicated considerably higher concentrations for subgroups of children compared to adults or the elderly for PFHS and, to a lesser extent, M570 (Figure 5). Whereas 95% of the adult and elderly population data had serum PFHS

Table 4. Estimates (ppb) of upper tolerance limits and their upper 95th percent confidence limits for fluorochemicals in children's serum samples

Fluorochemical	Upper tolerance limit	Estimate	Upper 95th percent confidence limit
PFOS	90%	71	75
	95%	89	97
	99%	141	217
PFOA	90%	8	9
	95%	10	11
	99%	17	20
PFHS	90%	34	39
	95%	65	81
	99%	156	416
PFOSAA	90%	9	9
	95%	10	11
	99%	18	21
M570	90%	7	8
	95%	12	15
	99%	26	38
TOF	90%	78	92
	95%	112	125
	99%	203	482

distributions less than 10 ppb, only 73% of the children were below this concentration. Furthermore, only 1 of 645 adults and 1 of 238 elderly blood donors had serum PFHS concentrations greater than 30 ppb (Olsen *et al.* 2003b, 2004) compared to 67 (11%) of the 598 children serum samples. These 67 children were disproportionately male (64%) compared to the overall study population (50%) but were not significantly different by age. For M570, only 4 adults (0.6%) and no elderly subjects had M570 concentrations $\geq$10 ppb (Olsen *et al.* 2003b, 2004) compared to 37 (6%) of the 598 children. As with PFHS, these 37 children were disproportionately male subjects (62%). Twenty-nine (78%) of these 37 children were under the age of six compared to an expected percentage of 53% based on the study population distribution. PFHS and M570 were poorly correlated ($r = .04$) in the present study and the correlation was only modestly greater when restricted to the 67 children with the highest PFHS concentrations ($r = 0.19$).

DISCUSSION

An important reason for undertaking the present study was to compare children's serum fluorochemical concentrations to those reported in adults and the elderly, which have used the same analytical methodology (Olsen *et al.* 2003b, 2004). Serum concentrations of chemicals may be different between children and adults for several reasons (Bearer 1995; Cohen-Hubal *et al.* 2000): 1) children's activity patterns may

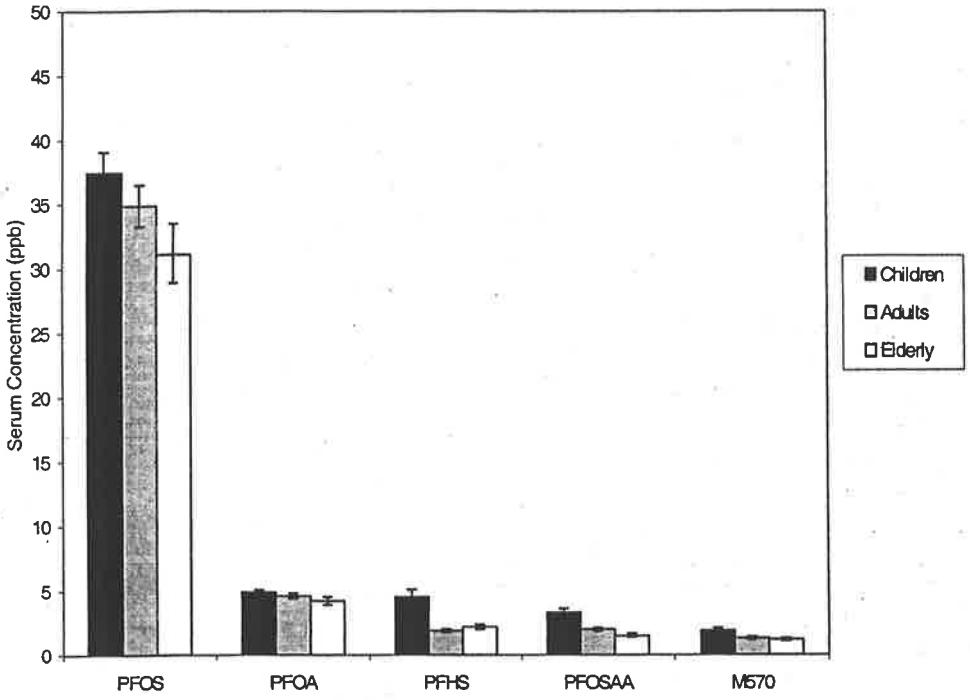

Figure 4. Geometric mean serum concentrations and 95th percent confidence intervals for PFOS, PFOA, PFHS, PFOSAA, and M570 in children (present study), adult (Olsen *et al.* 2003b) and elderly (Olsen *et al.* 2004) populations.

be different; for example, playing on the floor may lead to more exposure to carpeted surfaces; 2) children have a larger surface area relative to body weight than do adults, which may increase the opportunity for dermal absorption; 3) children have higher basal metabolic rates and energy requirements and therefore different pharmacokinetics could apply; and/or 4) children may have greater relative exposures to environmental contaminants in air and food than adults.

The data suggest different exposure patterns may exist based on the subgroup of children that had higher serum concentrations of PFHS and M570 than adults. Exposure to N-MeFOSE (which can be oxidized to M570) and PFHS may occur through contact with treated carpet, which could have included mill and/or post-mill applications. Dermal absorption of both MeFOSE and PFHS is possible (Reed 2002a). One could speculate that children's activity and exposure patterns (e.g., playing on carpeted floors) might account for some of the difference in PFHS and M570 values between this subgroup of children and adults. Children in this sample whose data more mirror the adult sera data may have less exposure despite having the same activity patterns as other children.

Despite the different upper level distributions between PFHS and M570 in the children compared to adult and elderly populations, there appeared to be similar

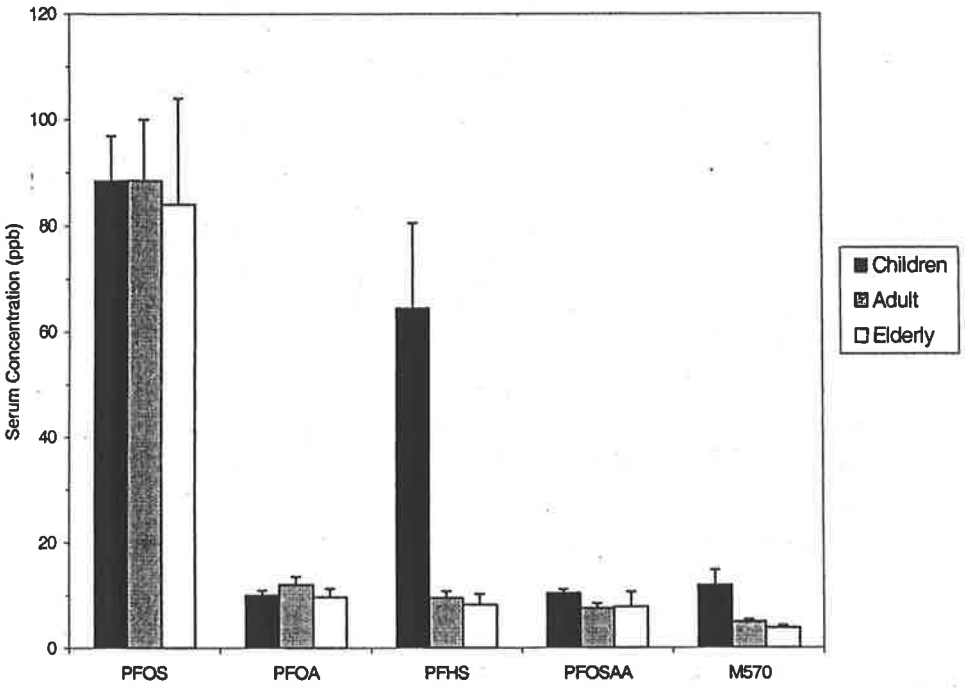

Figure 5. Estimates of the 95th percentile (upper tolerance limit) and 95th percent upper confidence limit for serum concentrations of PFOS, PFOA, PFHS, PFOSAA, and M570 in children (present study), adult (Olsen *et al.* 2003b) and elderly (Olsen *et al.* 2004) populations.

distributions between the three populations for PFOSAA as the upper bound of the mean 95% tolerance limit did not substantially differ (Figure 5). PFOSAA is an oxidation production associated with the N-EtFOSE–related paper and packaging protective treatments.

As was reported in the adult and elderly population studies (Olsen *et al.* 2003b, 2004), we observed a strong correlation between PFOS and PFOA in the children's serum. PFOS has been measured in human populations and in samples of wildlife tissue (Giesy and Kannan 2001; Kannan *et al.* 2001a, b, 2002a, b; Hansen *et al.* 2001; 3M Company 2003), whereas serum PFOA concentrations have been consistently quantified (i.e., measured above the LLOQ) primarily only in humans, although reported occasionally in wildlife (Kannan *et al.* 2002c). In many cases, the LLOQ for wildlife determination of PFOA was above the LLOQ for human determination. An association between PFOS and PFOA is of interest because PFOA cannot convert directly from PFOS (or vice versa). Whether this statistical association is due to the presence of PFOA as a by-product in POSF-based production, as a metabolite of these materials, the use of PFOA itself, or to other environmental or consumer products unrelated to POSF-based materials (e.g., higher carbon chain telomer alcohols) remains to be determined. Any of these explanations, coupled with the suspected

long serum half-life (i.e., years) in humans as reported in preliminary observation by Burris *et al.* (2002), could explain the correlation between PFOS and PFOA. Serum PFHS also appears to have a long half-life of elimination in humans (i.e., years) as reported by Burris *et al.* (2002).

Methodologically, our procedures allowed for a nonbiased sampling of the original study database. Fifty-three percent of all stored samples were analyzed including all samples for children less than five years of age. Our study included only children with group A streptococcal infections. Noninvasive streptococcal A infection is quite prevalent, with more than ten million cases occurring annually in the United States and it is one of the most common bacterial infections of school-aged children (CDC 2002). Because there was no exposure component to our investigation, the study was methodologically weaker than regional or national exposure assessments and biomonitoring surveys that have occurred for other environmental chemicals, which have incorporated probability-based sampling techniques (Quackenboss *et al.* 2000; CDC 2003a). Additional assessments of the distribution of serum PFOS and other fluorochemical concentrations in non-occupationally exposed populations, which may incorporate exposure assessments as well as biomonitoring efforts, are now under consideration by the National Center for Environmental Health at the Centers for Disease Control and Prevention (CDC 2003).

Substantial literature exists regarding the toxicology of PFOS (3M Company 2003; OECD 2002) and PFOA (Kennedy *et al.* 2003). Results from several repeat-dose toxciological studies consistently demonstrate that the liver is the primary target organ with an apparent threshold for the toxic effects of PFOS that can be expressed in terms of cumulative dose or body burden (Butenhoff and Seacat 2001; Butenhoff *et al.* 2002a; Seacat *et al.* 2002a, b, 2003; Lau *et al.* 2003; Thibodeaux *et al.* 2003). Lowered serum total cholesterol appeared to be a consistent early finding, with cumulative toxicity resulting in metabolic wasting and ultimately death in laboratory animals exposed to the high doses. Two-year feeding studies of PFOS and N-EtFOSE in rats produced a modest, largely benign liver tumor response in the high dose groups, which likely occurred through nongenotoxic mechanisms (Seacat *et al.* 2002b; Thomford *et al.* 2002). Neither PFOS or N-EtFOSE were found to be selective teratogens (Case *et al.* 2001), but PFOS did reduce postnatal survival and body weight gains in dams and pups in two-generation studies of mice and rats (Butenhoff *et al.* 2002a; Lau *et al.* 2003; Thibodeaux *et al.* 2003). Hypothyroxemia (reduced serum thyroxin) has been reported in these animals, however, without a feedback increase in thyroid stimulating hormone (Lau *et al.* 2003). Nor was there evidence of thyroid toxicity or decrements in learning or memory acquisition in the rat or mouse. Cross fostering studies suggest both in utero and lactational pathways of exposure are possible (3M Company 2003). Both of these exposure pathways likely also exist in humans and could explain, in part, the serum concentrations observed for PFOS in very young children coupled with its long (years) serum elimination rate (Burris *et al.* 2002). After consideration of serum and liver PFOS concentrations associated with no-observed-adverse-effect levels and benchmark dose calculations from several

toxicological studies, a human serum concentration of 100 ppb PFOS (the upper bound estimate of the 95th percentile observed in the present study) was related to margins of exposure between 310 and 1550 depending upon the point of departure used in the analysis (3M Company 2003).

Sex and species differences exist in the elimination of PFOA (Hanhijarvi and Ylinen 1988; Johnson and Ober 1980; Kemper 2003). In addition to urinary excretion, biliary excretion and reabsorption of PFOA occurs (Johnson *et al.* 1984). In the primate, the terminal phase elimination half-life in serum for both sexes was approximately one month (Butenhoff *et al.* 2002b) but may be much longer (several years) in the human (Burris *et al.* 2002). Rats fed a diet of 300 ppm PFOA (daily dose of 15 mg/kg/day) had increased incidences of liver, Leydig cell, and pancreas acinar cell adenomas, which likely occurred via nongenotoxic mechanisms (Biegel *et al.* 2001). Hepatic toxicity, hypolipidemia, and abnormal hormone levels have not been associated with serum PFOA concentrations in APFO production workers whose serum levels have averaged 5 ppm with a range between 0.1 and 114 ppm (Gilliland and Mandel 1996; Olsen *et al.* 1998, 2000). A retrospective cohort mortality study of employees who worked for one year or longer in APFO production jobs did not report statistically significant increased standardized mortality ratios (SMR) for liver cancer (SMR = 0.6, 95% CI 0.0–3.3), cirrhosis of the liver (SMR = 0.7, 95% CI 0.2–1.8), or pancreas cancer (SMR = 1.4, 95% CI 0.5–3.1) (Alexander 2001).

The epidemiology and toxicology of the other fluorochemicals measured have not been studied to the same extent as PFOS and PFOA. Fluorochemical production workers were reported to have a geometric mean serum PFHS concentration of 180 ppb (95% CI 1145–223) with a range of values from 5 to 1,880 ppb (Olsen *et al.* 2003d). Toxicologically, male rats had reductions in serum cholesterol at 0.3 mg/kg/day, liver weight increase, and hepatocellular hypertrophy as well as hepatocellular and thyroid follicular cell hyperplasia at doses of 3 and 10 mg/kg/day PFHS for 42 days (Reed 2002b). By contrast, no effects were observed in female rats dosed 14 days prior to mating and through gestation and lactation at doses as high as 10 mg/kg/day for PFHS. No effects were observed in fertility and mating parameters or in the offspring of PFHS-treated male and female rats through weaning. For M570, the fluorochemical production workers had a geometric mean serum concentration of 81 ppb (95% CI 67–98) with a range of values from 8 to 992 ppb (Olsen *et al.* 2003d). In a subchronic dietary study in which male and female rats were fed up to 100 ppm in their diet for 13 weeks, N-MeFOSE produced effects similar to those by subchronic dietary dosing with PFOS (Seacat *et al.* 2000).

CONCLUSION

Serum concentrations of PFOS were comparable whether measured in children (ages 2–12), adults (ages 20–69), or the elderly (ages 65–96) from samples collected in the United States. Additional information will be helpful to more fully understand the prevalence and distribution of these fluorochemicals in representative samples of the civilian population in the United States and other countries. The

similarity observed across age and gender will be useful in human risk characterization because serum PFOS concentrations likely reflect cumulative human exposure. Such similarity for PFOS (and PFOA), however, was not observed for PFHS, and to a lesser extent, M570. The reasons for these dissimilarities remain to be determined. Different exposure and activity patterns between children and adults should be considered.

ACKNOWLEDGEMENTS

3M provided funding for this study. 3M is a diversified technology company with business positions in consumer and office; display and graphics; electronics and telecommunications; health care; industrial; safety, security, and protection services; and transportation. Dr. Edward Kaplan (University of Minnesota Department of Pediatrics) provided the children's serum samples. Laboratory analysis of the seven fluorochemicals was conducted at Tandem Labs (formerly Northwest Bioanalytical, Inc) and included the following individuals: Ann Hoffman, Connie Sakashita, Patrick Bennett, Dr. Rodger Foltz, Suzanne Newman, and Emily Yardimici. The authors also thank Kimberly Young (3M Medical Department) for her contributions.

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